REGENERATION AND REORGANIZATION IN UROLEPTUS MOBILIS FOLLOWING INJURY BY INDUCED ELECTRIC CURRENTS

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Introduction

The interesting investigations of Calkins (1911), Young (1922), and more recently, Dembowska (1925), Taylor (1928), and Reynolds (1932) have demonstrated quite conclusively that regeneration in the Infusoria is accompanied by profound protoplasmic changes. These changes are evidenced by a dedifferentiation and redifferentiation of the regenerating protoplasm comparable to that occurring during binary fission. Thus, it has been shown that the locomotor organelles in the regenerating pieces are entirely absorbed and a complete new set redifferentiated in the reformed organism.

Most investigations along these lines have taken the form of merotomy experiments involving transections, incisions, and excisions of various sorts. This gives rise to the interesting question of whether or not more drastic types of injury or mutilation would result in regeneration of the injured organism. And, if regeneration does occur, is its method of reorganization similar to that observed in other types of mutilations in the ciliates? Relatively few experiments, however, have been performed to test the effects of more drastic mutilations on regeneration in these animals. It is well known that electric currents, under certain intensities, can produce severe injuries to cells and protoplasm in general. The following investigation was designed to determine whether ciliates, injured and mutilated by rapidly induced electric currents, would undergo regeneration and reorganization comparable to that following other reported types of mutilations.

Although careful observations were made on abnormalities in the movements of the organisms (including effects on the motile organelles), vacuolization, and disintegration, attention was particularly centered on the nuclei and on the subsequent behavior of these important cellular constituents.

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Materials and Methods

Uroleptus mobilis was found to be well adapted for studies of this nature, and was used throughout this experiment. An abundant culture was obtained through the kindness of Dr. H. R. Halsey of Columbia University. Sub-cultures were made and maintained in a medium of hay infusion to which was added some "Pablum" and powdered rice. In the procedure described below, only vigorous and flourishing cultures were used, and from these were selected individuals that were not in the process of division. The technique employed throughout was similar to that described in an earlier report (Tittler, 1937). The source of electricity was supplied by two dry-cell batteries in series, attached to an inductorium (Harvard Apparatus Company), which was set for rapidly induced (tetanizing) shocks. A simple key was introduced into the circuit for making and breaking contact. Platinum electrodes from the secondary coil of the inductorium were led into opposite sides of a small "tetanizing" chamber. This latter piece of apparatus consisted simply of an ordinary 1½ by 3-inch glass slide, around the sides of which were built walls of paraffin, arranged in such a fashion as to form a glass-bottomed trough measuring 2 by 4 cm. with a depth of 1.4 cm. The platinum electrodes were fixed at a constant distance of 2.5 cm.

The organisms to be exposed were introduced into the rectangular "tetanizing" chamber, which was then placed on the stage of a binocular microscope. This arrangement permitted free observation of the organisms during and following periods of stimulation. The intensity of the current was controlled by sliding the secondary coil of the inductorium forward or backward as desired. Individual organisms were removed and isolated during or following exposure for future observation. At regular intervals after exposure, *Uroleptus* individuals were fixed on coverslips, stained, and mounted. For permanent preparations, Gilson-Carnoy's and Schaudinn's fluid with acetic acid were employed, followed by Heidenhain's haematoxylin, Mayer's haemalum, and the Feulgen nucleal reaction.

Under the conditions of this experiment, a sixty-second exposure to the current was found to be sufficient to produce various degrees of injury in *Uroleptus*. Currents of greater duration proved lethal, and were not used.

Observations

General

The structure and life history of *Uroleptus mobilis* has been thoroughly presented by Calkins (1919). Consequently, a lengthy discus-

sion of its general morphology at different phases of its life cycle is not warranted. (For a complete critical review, the reader is referred to Calkins, 1933). As Calkins points out, the vegetative macronuclei, usually eight in number, are arranged in a linear fashion within the cell. Occasionally, individuals with nine to twelve macronuclei have been found. The micronuclei are minute and homogeneous and vary in number from two to six. In preparation for division, a typical nuclear cleft appears in each macronucleus, and in each cleft is found a single large granule. This latter structure together with some chromatin is thrown off and the remaining chromatin contents are distributed in the cytoplasm. Subsequently, these "freed" nuclei fuse and condense into a relatively small ellipsoidal and single nucleus. The latter divides two or three times before the cell divides, followed by a fourth division after the daughter cells have separated. The division of the micronucleus is not as complicated. Each divides mitotically and is represented by daughter halves in each of the daughter cells.

Apparently, as in other hypotrichs during binary fission, the motor organelles are resorbed and a new set reformed in each of the resulting individuals.

Under the influence of the induction current the normal movements of *Uroleptus* are considerably affected. The organisms are drawn towards the cathode of the break shocks, at the same time exhibiting a peculiar twisting, lashing movement of the tail region. In some instances a few individuals were seen to swim towards the anode, a phenomenon also observed by Austin (1927). As organisms neared the cathode, marked vacuolization of the cytoplasm occurred, followed by disruption and eventual disintegration of the cell bodies. It was interesting to note that in about 75 per cent of observed cases, as the induced shocks were passed through the culture, the animals were found to be affected and disrupted especially at that end which faced the anode, that is, at the posterior region. This gave rise to many deformed and stumpy animals with the peristomes still intact. During this period pieces of diverse sizes, manifesting various degrees of injury, were obtained. Some of these were isolated for observation. Others were fixed and stained for permanent records.

Figure 1, *A, B, C, D,* shows a few of these individuals fixed immediately after exposure. Different conditions are depicted, ranging from a small stunted fragment, lacking peristome and many macronuclei and micronuclei, to an organism only slightly affected.

Fragments of *Uroleptus,* mutilated by the current, reacted in the following manner. Immediately after removal, anterior pieces with frontal cirri and membranelles still intact, tended to spin in circles for

a time, but soon this spinning ceased and the pieces swam in their normal lively manner. Posterior pieces lacking a peristome, remained comparatively motionless for a few seconds and then swam in the customary fashion. These findings confirmed those of Jennings and Jamieson (1902) and others, who demonstrated that pieces of ciliates move in the same essential manner as the entire animal. During this time the wounded edges closed over and began to round out. In less than an

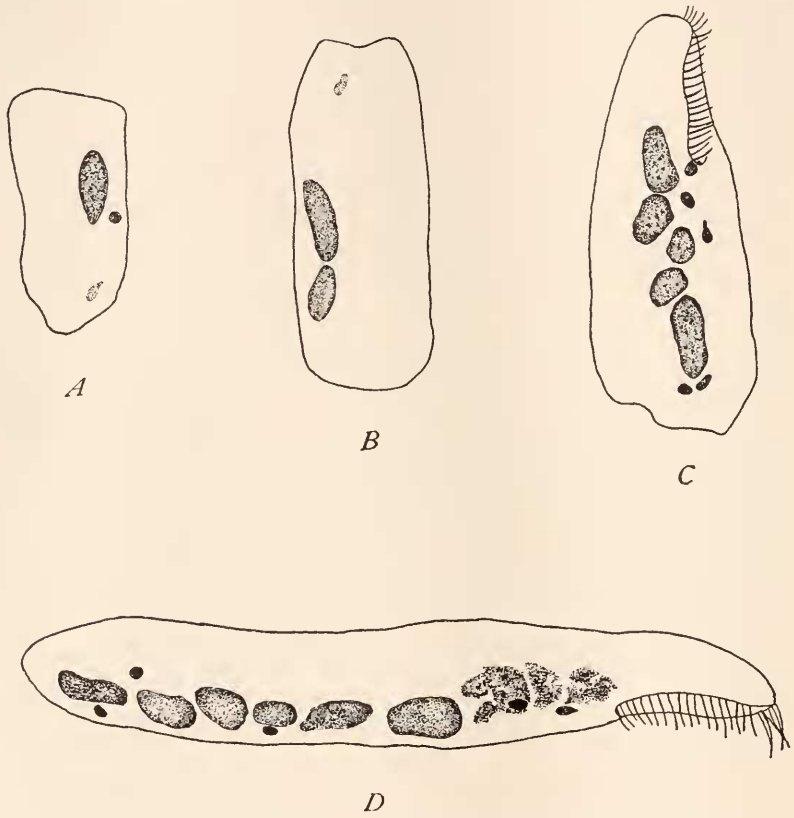

FIG. 1. Camera lucida drawings of material fixed immediately after exposure to the electric current. Cilia omitted.

A. Fragment from posterior region of organism, with one intact macronucleus and two micronuclei. One of the latter is faint and elongate. Peristome lacking. Such fragments do not regenerate. × 1157.

B. Fragment with two macronuclei and one micronucleus, the latter faint and ghost-like. × 1144.

C. Fragment from anterior region of organism. Peristome intact. × 1120.

D. Intact individual. The macronuclei at the anterior region have been disrupted and flattened by the electric current. Remaining macronuclei are unaffected. × 1168.

hour the movements of the pieces slowed down and each was seen to be completely dedifferentiated. Shortly after this it became apparent that a redifferentiation and reorganization was going on within them. It was difficult to observe all the changes which took place in anterior pieces during this protoplasmic reorganization, but as far as could be determined, the cirri and membranelles were withdrawn and a new set developed in the reformed individuals. In posterior pieces, a new peristome appeared, and lost organelles were completely restored. Within three to eight hours each fragment had regenerated its missing structures. At the end of thirty-four hours most of the regenerated individuals were in the process of division. Variations in the time intervals for complete regeneration following injury were probably due to the degree of injury and to whether the regenerating pieces were from anterior or posterior regions of the animal. Thus, it was found that smaller pieces regenerated more slowly than larger ones and often required two days for growth and internal reorganization before dividing. Similarly, regeneration of anterior pieces began earlier than posterior ones.

Nuclei

In material fixed before visible disintegration had occurred, it was evident that the macronuclei in the affected region of the cell were beginning to break up and the nuclear membrane disrupted. The chromatin granules tended to disperse somewhat, resulting in flattened, amorphous nuclei (Fig. 1, *D*). The remaining nuclei were still intact. The micronuclei were elongated and some appeared pale and ghost-like as though they were being absorbed.

In animals stained three to eight hours after exposure the nuclei were found to be in various stages of reorganization. It was evident from a study of fixed stages that this involved the same sort of nuclear reorganization as that which regularly occurs during binary fission in *Uroleptus*. Nuclear clefts appeared and the macronuclei began to fuse with adjacent nuclei and condense into a relatively small division nucleus (Fig. 2, *A, B*). This constricted rapidly and divided into the eight macronuclei characteristic of normal individuals, without the division of the cell (Fig. 2, *C, D, E*). The micronuclei underwent mitotic division, and at times as many as eight of them were found in one cell. However, at the completion of reorganization some of these were absorbed, the number remaining never exceeding six. Occasional individuals were seen to possess ten macronuclei, but the normal nuclear complex was usually restored after normal division. Apparently, regardless of the degree of injury, most fragments possessing two or

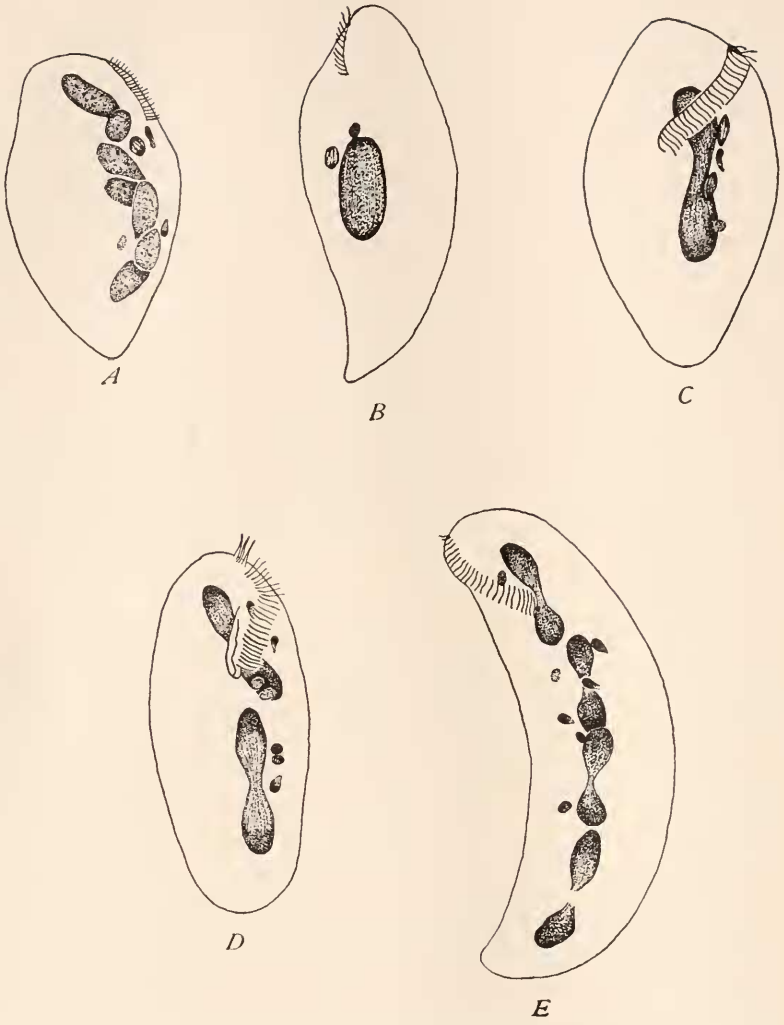

FIG. 2. Camera lucida drawings of individuals fixed three to five hours after exposure to the current. Nuclei in various stages of reorganization. Cilia omitted.

A. Macronuclei beginning to coalesce. One micronucleus in mitosis. × 1052.

B. Later stage showing final fusion of macronuclei into compact division nucleus. × 1087.

C. Division nucleus beginning to constrict into two. Note linear arrangement of chromatin. Two micronuclei in mitosis. × 945.

D. Later stage showing further constriction of the nucleus. × 1001.

E. Individual in late phase of nuclear reorganization, showing final constriction of the nucleus to form eight macronuclei. Division of the cell does not occur during these reorganization changes. × 1013.

more macronuclei and at least one micronucleus were able to reorganize and regenerate. In some instances, smaller pieces, although they contained sufficient nuclear material, were unable to regenerate completely, and perished. Their failure to do so was probably due to an insufficient amount of cytoplasm in which to undergo a reorganization.

In all observed cases of functional regeneration, the stained portions indicated that some macronuclear and micronuclear material had been included in the living fragment. In addition, specimens of these regenerated individuals immediately after division exhibited a perfectly normal nuclear condition.

Discussion

The results here reported on the effects of induced currents on injury and regeneration in *Uroleptus mobilis* bear out the findings of other investigators that mutilations in the hypotrichous ciliates are followed by reorganization changes comparable to those occurring at the time of division. Thus, it has been demonstrated that even after severe degrees of injury and partial cytolysis inflicted by electric currents, *Uroleptus* organisms were able to regenerate and undergo profound reorganization changes involving a dedifferentiation and redifferentiation of the external motor organelles, such as was reported by Calkins (1911) and Young (1922) in *Uronychia,* Dembowska (1925) in *Stylonychia,* Taylor (1928) in *Uronychia uncinata,* Reynolds (1932) in *Oxytricha,* and others.

These results are perhaps more significant because they shed further light on the behavior of the nuclei during regeneration. Balbiani (1891), working with *Stentor,* was probably one of the earliest investigators to point to the interesting fact that the macronucleus goes through precisely the same changes in regeneration as in fission. Johnson (1893) subsequently confirmed this. More recently, Young (1926) found that when *Stylonychia* was transected, the macronucleus and micronucleus in each of the resulting pieces divided into two to restore the normal nuclear complex. However, although later workers were concerned with the respective rôles of the nuclei in regeneration, their interests seemed to lie largely with the problem of the relation of the nuclear content of pieces with regard to functional regeneration—a subject which will be briefly dealt with below. This seems rather surprising in view of the fact that it has become increasingly evident that the behavior of the nuclei in the Infusoria is indicative of deep-seated reorganization changes going on within the cell.

In *Uroleptus* it is apparent that during regeneration the nuclei, particularly the macronuclei, undergo changes which are entirely comparable to those occurring during normal division in this organism (see

Calkins, 1919). This observation is significant because of its bearing on Taylor's statement (1928) that "There remains, too, the study of internal organelles, such as fibrils and nuclei, which may very possibly undergo changes during regeneration similar to those described above for external motor organs." It would appear, therefore, that in *Uroleptus,* at any rate, the nuclei do undergo such changes during regeneration.

Results obtained from the various merotomy experiments in the Protozoa are in complete agreement with the belief that some nuclear material is necessary for complete regeneration. (For a critical review of the evidence, Reynold's paper (1932) may be consulted.) Gruber's observation (1886) on *Stentor* is the only recorded exception. He found that an enucleated piece from a dividing individual, in which the new peristome had already appeared, was able to develop into a perfect organism, but he failed to give an account of its later history.

Evidently, in most cases, complete functional regeneration seemed to be dependent upon the presence of both macronuclear and micronuclear material. This was found to be true for *Euplotes* (Taylor, 1923, Reynolds, 1932), *Spathidium* and *Blepharisma* (Moore, 1924), and others. Calkins (1911) and Young (1922) were able to show that in *Uronychia* some pieces could regenerate with only macronuclear material at certain periods after the division of the organism, but Young pointed out that these became abnormal, so that for perfect regeneration the micronucleus was necessary. Reynolds (1932), working with an amicronucleate race of *Oxytricha fallax,* found that these organisms were able to regenerate their morphological parts and become normal in every respect. She concluded, however, that the macronucleus of this race was really an amphinucleus.

Uroleptus yielded similar results because, without exception, in all cases where functional regeneration was completed, it was found that some macronuclear and micronuclear material was present in the living fragment. Pieces devoid of such material were able to move in a normal way and even undergo partial regeneration but eventually they died.

Summary

1. *Uroleptus mobilis* organisms were subjected to injury and mutilation by rapidly induced electric currents to determine whether ciliates, drastically injured, would undergo regeneration and reorganization comparable to that following other types of mutilation.

2. Under the influence of the induction current most of the organisms moved abnormally, and were drawn towards the cathode of the break shocks. Disruption and disintegration of the cell bodies occurred,

usually, at the posterior region. This resulted in diverse pieces manifesting various degrees of injury.

3. Injured pieces retained their original polarity and moved in essentially the same manner as the entire animal.

4. Within three to eight hours after injury, each fragment had regenerated its missing structures, and at the end of thirty-four hours most of the regenerated individuals were in the process of division.

5. Regeneration was accompanied by profound protoplasmic reorganization changes comparable to those occurring at the time of division. Cortical organelles were withdrawn, and new sets developed in the reformed individuals. The macronuclei fused into a relatively small division nucleus followed by the rapid constriction of the latter into the eight macronuclei characteristic of normal individuals. The micronuclei divided mitotically.

6. The presence of both macronuclear and micronuclear material was necessary for complete functional regeneration in *Uroleptus*.

LITERATURE CITED

Austin, M. L., 1927. Studies on Uroleptus mobilis. II. The conditions necessary for conjugation. *Jour. Exper. Zoöl.*, **49**: 149.

Balbiani, E. G., 1891. Sur les régénérations successives du peristome comme caractère d'âge chez les Stentors et sur le rôle du noyau dans ce phénomène. *Zoöl. Anzeig.*, **14**: 312.

Calkins, G. N., 1911. Regeneration and cell division in Uronychia. *Jour. Exper. Zoöl.*, **10**: 95.

Calkins, G. N., 1919. Uroleptus mobilis Engelm. I. History of the nuclei during division and conjugation. *Jour. Exper. Zoöl.*, **27**: 293.

Calkins, G. N., 1933. The Biology of the Protozoa. Philadelphia. Second Edition.

Dembowska, W. S., 1925. Studien über die Regeneration von Stylonychia mytilus. *Arch. f. mikr. Anat. und Ent.*, **104**: 185.

Gruber, A., 1886. Beiträge zur Kenntniss der Physiologie und Biologie der Protozoen. *Ber. d. Naturf. Ges. Freiburg.*, **1**: 33.

Jennings, H. S., and C. Jamieson, 1902. Studies on reactions to stimuli in unicellular organisms. X. The movements and reactions of pieces of ciliate Infusoria. *Biol. Bull.*, **3**: 225.

Johnson, H. P., 1893. A contribution to the morphology and biology of the Stentors. *Jour. Morph.*, **8**: 467.

Moore, E. L., 1924. Regeneration at various phases in the life-history of Spathidium spathula and Blepharisma undulans. *Jour. Exper. Zoöl.*, **39**: 249.

Reynolds, M. E. C., 1932. Regeneration in an amicronucleate infusorian. *Jour. Exper. Zoöl.*, **62**: 327.

Taylor, C. V., 1923. Rôle of the micronucleus in Euplotes. *Anat. Rec.*, **26**: 376.

Taylor, C. V., 1928. Protoplasmic reorganization in Uronychia ucinata sp. nov. during binary fission and regeneration. *Physiol. Zoöl.*, **1**: 1.

Tittler, I. A., 1937. The effects of tetanizing shocks on Paramecium and Uroleptus with reference to injury and recovery. *Anat. Rec.*, **70**: Supplement No. 1: 125.

Young, D. B., 1922. A contribution to the morphology and physiology of the genus Uronychia. *Jour. Exper. Zoöl.*, **36**: 353.

Young, D. B., 1926. Nuclear regeneration in Stylonychia mytilus. *Biol. Bull.*, **51**: 163.